



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68	A2	(11) International Publication Number: WO 99/00517 (43) International Publication Date: 7 January 1999 (07.01.99)
<p>(21) International Application Number: PCT/GB98/01893</p> <p>(22) International Filing Date: 29 June 1998 (29.06.98)</p> <p>(30) Priority Data: 9713666.7 27 June 1997 (27.06.97) GB</p> <p>(71) Applicant (for all designated States except US): CAMBRIDGE UNIVERSITY TECHNICAL SERVICES LIMITED [GB/GB]; The Old School, Cambridge CB2 1TS (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): ARCHER, John, Anthony, Charles [GB/GB]; University of Cambridge Department of Genetics, Downing Street, Cambridge CB2 3EH (GB). SUMMERS, David, Keith [GB/GB]; University of Cambridge Department of Genetics, Downing Street, Cambridge CB2 3EH (GB). ROLAND, Hervé, Jacquiau [FR/GB]; University of Cambridge Department of Genetics, Downing Street, Cambridge CB2 3EH (GB). POWELL, Justin, Antoine, Christian [GB/GB]; University of Cambridge Department of Genetics, Downing Street, Cambridge CB2 3EH (GB).</p> <p>(74) Agents: KREMER, Simon, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>
<p>(54) Title: BIOSENSOR MATERIALS AND METHODS</p>		
<p>(57) Abstract</p> <p>Disclosed are methods for generating mycolic acid bacterial biosensors for particular analytes (especially industrial pollutants) by the use of innovative methods for isolating DNA encoding an inducible promoter which is induced in response to the specific analyte (and/or associated operon proteins), the methods generally comprising the steps of: (a) culturing a source of mycolic acid bacteria in a selective medium containing said specific analyte and being selective for oligotrophic bacteria; (b) identifying mycolic acid bacteria capable of subsisting on said medium, especially those which do not display catabolic repression; (c) extracting DNA from said mycolic acid bacteria; (d) incorporating said DNA into vectors, such as various shuttle vectors; (e) cloning said vector into a suitable host cell (which may be <u>E. coli</u> strain carrying one or more of the <u>mcrABC</u> <u>mrr</u> <u>hsdSRM</u> <u>recA</u> and <u>recO</u> mutations); (f) screening that host cell (or a second host cell which is preferably a corynebacterium) for said inducible promoter. The methods are exemplified by the isolation of the <u>R. corallina</u> <u>ohp</u> operon. Also disclosed are associated materials, e.g. media, vectors, nucleic acid probes for performing the invention, and biosensors produced by the methods of the invention plus methods of use of the same.</p>		